

AMENDMENTS TO THE CLAIMS:

The following Listing of Claims replaces all prior Listings and versions of claims in the above-identified application.

Listing of Claims

1. (Currently Amended) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:
 - a) culturing in a fermentation medium a bacterium or yeast microorganism which comprises at least one genetic modification that results in the increased expression of a bacterial or yeast increases the activity of glucosamine-6-phosphate acetyltransferase; and
 - b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.
2. (Currently Amended) The method of Claim 1, wherein the genetic modification that results in the increased expression of a bacterial or yeast glucosamine-6-phosphate acetyltransferase is an increase in the copy number of a nucleic acid encoding the bacterial or yeast glucosamine-6-phosphate acetyltransferase or the use of a promoter that gives higher levels of expression than the native promoter. ~~to increase the activity of glucosamine-6-phosphate acetyltransferase provides a result selected from the group consisting of: increased enzymatic activity of glucosamine-6-phosphate acetyltransferase; overexpression of glucosamine-6-phosphate acetyltransferase by the microorganism; reduced N-acetylglucosamine-6-phosphate product inhibition of the glucosamine-6-phosphate acetyltransferase; and increased affinity of glucosamine-6-phosphate acetyltransferase for glucosamine-6-phosphate.~~
3. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast microorganism is transformed with at least one recombinant nucleic acid molecule

comprising a nucleic acid sequence encoding the bacterial or yeast glucosamine-6-phosphate acetyltransferase.

4. (Currently Amended) The method of Claim 3, wherein the nucleic acid molecule further comprises a non-native promoter, sequence encoding a glucosamine-6-phosphate acetyltransferase has at least one genetic modification which increases the enzymatic activity of the glucosamine-6-phosphate acetyltransferase.

5-6. (Canceled)

7. (Currently Amended) The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least 95% about 70% identical to the [[an]] amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has acetyltransferase enzymatic activity.

8. (Currently Amended) The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has the [[an]] amino acid sequence selected from the group consisting of SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34.

9. (Previously Presented) The method of Claim 3, wherein expression of the recombinant nucleic acid molecule is inducible.

10. (Previously Presented) The method of Claim 9, wherein expression of the recombinant nucleic acid molecule is inducible by lactose.

11. (Currently Amended) The method of Claim 10, wherein the bacterium or yeast microorganism further comprises a genetic modification to reduce inhibition of transcription induction by lactose.

12. (Previously Presented) The method of Claim 11, wherein the genetic modification comprises a partial or complete deletion or inactivation of a gene encoding a LacI repressor protein.

13. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast microorganism further comprises at least one genetic modification that increases the activity of a bacterial or yeast glucosamine-6-phosphate synthase[[.]], wherein the at least one genetic modification is increased expression of the glucosamine-6-phosphate synthase or at least one mutation or deletion in the amino acid sequence of the glucosamine-6-phosphate synthase that results in a reduced product inhibition of the glucosamine-6-phosphate synthase activity as compared to the wild-type glucosamine-6-phosphate synthase.

14. (Currently Amended) The method of Claim 13, wherein the bacterium or yeast microorganism is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast [[the]] glucosamine-6-phosphate synthase.

15-16. (Canceled)

17. (Currently Amended) The method of Claim 14, wherein the bacterial or yeast glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least 95% about 70% identical to the [[an]] amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has synthase enzymatic activity.

18. (Currently Amended) The method of Claim 14, wherein the bacterial or yeast glucosamine-6-phosphate synthase comprises the [[an]] amino acid sequence

selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20.

19. (Currently Amended) The method of Claim 14, wherein the bacterial or yeast glucosamine-6-phosphate synthase has a modification to reduce product inhibition of the glucosamine-6-phosphate synthase as compared to the wild-type glucosamine-6-phosphate synthase.

20. (Currently Amended) The method of Claim 19, wherein the bacterial or yeast glucosamine-6-phosphate synthase comprises the [[an]] amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

21. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast microorganism further comprises at least one partial or complete deletion of an endogenous gene encoding a glucosamine-6-phosphate deaminase in the bacterium or yeast genetic modification that decreases the activity of glucosamine-6-phosphate deaminase in the bacterium or yeast.

22. (Canceled)

23. (Currently Amended) The method of Claim 13, wherein the bacterium or yeast microorganism further comprises at least one partial or complete deletion of an endogenous gene encoding a glucosamine-6-phosphate deaminase in the bacterium or yeast genetic modification that decreases the activity of glucosamine-6-phosphate deaminase in the bacterium or yeast.

24. (Canceled)

25. (Previously Presented) The method of Claim 1, wherein the step of culturing includes the step of maintaining the carbon source at a concentration of from about 0.5% to about 5% in the fermentation medium.

26. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising yeast extract.

27. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising a carbon source selected from the group consisting of glucose, fructose, a pentose sugar, lactose and gluconic acid.

28. (Previously Presented) The method of Claim 27, wherein the pentose sugar is selected from the group consisting of ribose, xylose, and arabinose.

29. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and ribose.

30. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and gluconic acid.

31. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a temperature of from about 25°C to about 45°C.

32. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at about 37°C.

33. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4 to about pH 7.5.

34. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 6.7 to about pH 7.5.

35. (Previously Presented) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4.5 to about pH 5.

36. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast is a bacterium. ~~microorganism is selected from the group consisting of bacteria and fungi.~~

37. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast is a yeast. ~~microorganism is selected from the group consisting of bacteria and yeast.~~

38. (Currently Amended) The method of Claim 36[[1]], wherein the bacterium ~~microorganism~~ is a bacterium from a genus selected from the group consisting of: *Escherichia, Bacillus, Lactobacillus, Pseudomonas* and *Streptomyces*.

39. (Currently Amended) The method of Claim 36[[1]], wherein the bacterium ~~microorganism~~ is a bacterium from a species selected from the group consisting of: *Escherichia coli, Bacillus subtilis, Bacillus licheniformis, Lactobacillus brevis, Pseudomonas aeruginosa* and *Streptomyces lividans*.

40. (Currently Amended) The method of Claim 37[[1]], wherein the yeast ~~microorganism~~ is a yeast from a genus selected from the group consisting of: *Saccharomyces, Candida, Hansenula, Pichia, Kluveromyces*, and *Phaffia*.

41. (Currently Amended) The method of Claim 37[[1]], wherein the yeast ~~microorganism~~ is a yeast from a species selected from the group consisting of: *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Candida albicans*,

Hansenula polymorpha, Pichia pastoris, P. canadensis, Kluyveromyces marxianus and *Phaffia rhodozyma*.

42-44. (Canceled)

45. (Currently Amended) The method of Claim 1[[44]], wherein the bacterium or yeast microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast [[the]] phosphoglucoisomerase.

46. (Currently Amended) The method of Claim 45[[44]], wherein the phosphoglucoisomerase comprises the [[an]] amino acid sequence of SEQ ID NO:105.

47. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast microorganism further comprises a partial or complete deletion or inactivation of phosphofructokinase in the bacterium or yeast microorganism.

48. (Canceled)

49. (Currently Amended) The method of Claim 1[[48]], wherein the bacterium or yeast microorganism has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast [[the]] glutamine synthetase.

50. (Currently Amended) The method of Claim 49[[48]], wherein the glutamine synthetase comprises the [[an]] amino acid sequence of SEQ ID NO:89.

51. (Canceled)

52. (Currently Amended) The method of Claim 1[[51]], wherein the bacterium

or yeast microorganism has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast [[the]] glucose-6-phosphate dehydrogenase.

53. (Currently Amended) The method of Claim 52[[51]], wherein the glucose-6-phosphate dehydrogenase comprises the [[an]] amino acid sequence of SEQ ID NO:95.

54. (Currently Amended) The method of Claim 1, wherein the bacterium or yeast microorganism further comprises a partial or complete deletion ~~or inactivation~~ of at least one gene encoding an enzyme involved in genes encoding enzymes responsible for glycogen synthesis in the bacterium or yeast microorganism.

55. (Currently Amended) The method of Claim 54, wherein the at least one gene encoding an enzyme involved in genes encoding enzymes responsible for glycogen synthesis is selected from the group consisting of: comprise ADP-glucose pyrophosphorylase, glycogen synthase and a branching enzyme.

56. (Currently Amended) The method of Claim 1, wherein the at least one genetic modification does not inhibit the ability of the bacterium or yeast microorganism to metabolize galactose.

57. (Currently Amended) The method of Claim 1, wherein the step of collecting comprises recovering an intracellular product from the bacterium or yeast microorganism selected from the group consisting of: intracellular glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine and glucosamine or recovering an extracellular product from the fermentation medium selected from the group consisting of: glucosamine and N-acetylglucosamine.

58. (Currently Amended) The method of Claim 1, further comprising a step selected from the group consisting of:

- a) purifying a product selected from the group consisting of glucosamine and N-acetylglucosamine from the fermentation medium;
- ~~b) recovering a product selected from the group consisting of glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate from the microorganism;~~
- b) [[c]]] dephosphorylating a product selected from the group consisting of glucosamine-6-phosphate and glucosamine-1-phosphate to produce glucosamine; [[and]]
- c) [[d]]] dephosphorylating a product selected from the group consisting of N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce N-acetylglucosamine; and
- d) [[e]]] treating a product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce a glucosamine product selected from the group consisting of: glucosamine, glucosamine-6-phosphate and glucosamine-1-phosphate.

59. (Currently Amended) The method of Claim 58[[54]], wherein step (d) [[(e)]] comprises hydrolyzing the product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate, under acid and heat conditions or by enzymatic deacetylation.

60. (Previously Presented) The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by precipitating N-acetylglucosamine-containing solids from the fermentation broth.

61. (Previously Presented) The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by crystallizing N-

acetylglucosamine-containing solids from the fermentation broth.

62-206. (Canceled)

207. (Currently Amended) A method to produce glucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a bacterium or yeast ~~microorganism~~ which has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast glucosamine-6-phosphate synthase, wherein expression of the recombinant nucleic acid molecule is controlled by a lactose induction, and wherein the step of culturing comprises:
 - i) growing the bacterium or yeast ~~microorganism~~ in the fermentation medium comprising glucose as a carbon source at a pH of from about pH 4.5 to about pH 7 and at a temperature of from about 25°C to about 37°C;
 - ii) inducing transcription of the nucleic acid sequence molecule by addition of lactose to the fermentation medium ~~in the absence of adding additional glucose to the medium~~;
 - iii) fermenting the bacterium or yeast ~~microorganism~~ after step (ii) in the presence of glucose at a pH of from about 4.5 to about 6.7 and at a temperature of from about 25°C to about 37°C; and
- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate and glucosamine.

208. (Currently Amended) The method of Claim 207, wherein a source of trace elements is added to step (iii) ~~of fermenting~~.

209. (Previously Presented) The method of Claim 208, wherein the trace

elements include iron.

210. (Currently Amended) The method of Claim 207, wherein step (i) [[(ii)]] comprises growing the bacterium or yeast microorganism in the fermentation medium comprising glucose as a carbon source at a pH of about pH 6.9.

211. (Currently Amended) The method of Claim 207, wherein step (iii) comprises fermenting the bacterium or yeast microorganism after step (ii) in the presence of glucose at a pH of from about 4.5 to about 5.

212. (Currently Amended) The method of Claim 207, wherein step (iii) comprises fermenting the bacterium or yeast microorganism after step (ii) in the presence of glucose at a pH of about 6.7.

213-217. (Canceled).

218. (Currently Amended) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a bacterium or yeast microorganism that expresses:
 - i) a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate acetyltransferase; and
 - ii) a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase, wherein the bacterial or yeast glucosamine-6-phosphate synthase is selected from: (1) a bacterial or yeast glucosamine-6-phosphate synthase that has a modification to reduce at least one amino acid substitution that reduces product inhibition of the glucosamine-6-phosphate synthase as compared to the wild-type glucosamine-6-phosphate synthase, and (2) a naturally occurring bacterial

or yeast glucosamine-6-phosphate synthase that has less product inhibition than the endogenous glucosamine-6-phosphate synthase; and
~~wherein the microorganism comprises a partial or complete deletion or inactivation of phosphofructokinase; and~~

- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

219. (Currently Amended) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a bacterium or yeast microorganism that expresses:
 - i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase that has an amino acid sequence that is at least about 95% identical to SEQ ID NO:30 and has glucosamine-6-phosphate acetyltransferase enzymatic activity; and
 - ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase that has an amino acid sequence that is at least about 95% identical to the amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14, SEQ ID NO:6 and has glucosamine-6-phosphate synthase enzymatic activity; and
~~wherein the microorganism comprises a partial or complete deletion or inactivation of phosphofructokinase; and~~
- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

220. (Currently Amended) The method of Claim 219, wherein the glucosamine-6-phosphate acetyltransferase has the [[an]] amino acid sequence of SEQ ID NO:30.

221. (Currently Amended) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has the [[an]] amino acid sequence of SEQ ID NO:6.

222. (Currently Amended) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium an *E. coli* that expresses:
 - i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase that has the [[an]] amino acid sequence of SEQ ID NO:30; and
 - ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase that has the [[an]] amino acid sequence of SEQ ID NO:6; and
~~wherein the *E. coli* comprises a partial or complete deletion or inactivation of *pfkA*; and~~
- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

223. (Currently Amended) The method of Claim 229 [[219]], wherein the *E. coli* further comprises a partial or complete deletion ~~or inactivation~~ of *nagA*, *nagB*, and *nagE*.

224. (Currently Amended) The method of Claim 229 [[219]], wherein the *E. coli* further comprises a partial or complete deletion ~~or inactivation~~ of *manXYZ*.

225. (Currently Amended) The method of Claim 229 [[219]], wherein the

recombinant nucleic acid molecules of (a)(i) and (a)(ii) are inducible by lactose or galactose.

226. (Currently Amended) The method of Claim 229 [[219]], wherein the step of culturing is performed in a fermentation medium comprising glucose and fructose.

227. (New) The method of claim 218, wherein the bacterium or yeast further comprises a partial or complete deletion of phosphofructokinase.

228. (New) The method of claim 219, wherein the bacterium or yeast further comprises a partial or complete deletion of phosphofructokinase.

229. (New) The method of claim 222, wherein the *E. coli* further comprises a partial or complete deletion of *pfkA*.

230. (New) The method of Claim 228, wherein the bacterium or yeast further comprises a partial or complete deletion of N-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, and N-acetyl-glucosamine-specific enzyme II^{Nag}.

231. (New) The method of Claim 228, wherein the bacterium or yeast further comprises a partial or complete deletion of mannose transporter EIIM,P/III^{Man}.

232. (New) The method of Claim 228, wherein the recombinant nucleic acid molecules of (a)(i) and (a)(ii) are inducible by lactose or galactose.

233. (New) The method of Claim 228, wherein the step of culturing is performed in a fermentation medium comprising glucose and fructose.

234. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:4.

235. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:6.

236. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:8.

237. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:10.

238. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:12.

239. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence that is at least 95% identical to SEQ ID NO:14.

240. (New) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

a) culturing in a fermentation medium a bacterium or fungus which comprises at least one genetic modification that results in the increased expression of a bacterial or fungal glucosamine-6-phosphate acetyltransferase; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

241. (New) The method of claim 240, wherein the bacterium or fungus is a fungus.

242. (New) The method of claim 241, wherein the fungus is a yeast.